Claims

1. A method of constructing and selecting or screening a library of polynucleotide sequences of interest in filamentous fungal cells wherein the method comprises:

(a) transforming the fungal cells with a population of DNA vectors, wherein each vector comprises:

(i) a polynucleotide sequence encoding a fungal selection marker and a fungal replication initiating sequence wherein the marker and the replication initiating sequence do not vary within the population; and

(ii) a polynucleotide sequence of interest wherein the population of DNA vectors contains more than one variant of the polynucleotide sequence;

(b) cultivating the cells under selection pressure;

(c) selecting or screening for one or more transformants expressing a desired characteristic; and

(d) isolating the transformant(s) of interest.

2. The method according to claim 1, wherein the library of polynucleotide sequences of interest is prepared by random mutagenesis or naturally occurring allelic variations of at least one parent polynucleotide sequence having or encoding a biological activity or function of interest.

3. The method of claim 1 or 2, wherein the polynucleotide sequence encodes a polypeptide or is a control sequence; or wherein the polynucleotide sequence encodes a polypeptide or part thereof and further comprises a control sequence involved in the expression of the polypeptide or a part of such control sequence.

4. The method according to claim 3, wherein the polypeptide is a hormone, an enzyme, a receptor or a portion thereof, an antibody or a portion thereof, or a reporter, or a regulatory protein.

5. The method of claim 4, wherein the enzyme is an oxidoreductase, a transferase, a hydrolase, a lyase, an isomerase, or a ligase.

6. The method according to any of claims 4 or 5, wherein the enzyme is an aminopeptidase, amylase, carbohydrase, carboxypeptidase, catalase, cellulase, chitinase, cutinase, cyclodextrin glycosyltransferase, deoxyribonuclease, esterase, alpha-galactosidase, beta-galactosidase, glucoamylase, alpha-glucosidase, beta-glucosidase, invertase, laccase, lipase, mannosidase, mutanase, oxidase, a pectinolytic enzyme, peroxidase, phytase, polyphenoloxidase, a proteolytic enzyme, ribonuclease, transglutaminase, or xylanase.

7. The method according to claim 1 or 2, wherein the control sequence is an enhancer sequence, a leader sequence, a polyadenylation sequence, a propeptide sequence, a promoter, a replication initiation sequence, a signal sequence, a transcriptional terminator or a translational terminator.

8. The method of claim 7, wherein the promoter is derived from the gene encoding *Aspergillus oryzae* TAKA amylase, NA2-tpi and *Aspergillus niger* or *Aspergillus awamori* glucoamylase.

9. The method according to any of claims 1-8, wherein the selective marker is selected from the group of genes which encode a product which provides for resistance to biocide or

viral toxicity, resistance to heavy metal toxicity, or prototrophy to auxotrophs.

10. The method of claim 9, wherein the prototrophy is obtained from an enzyme selected from the group of metabolic pathways consisting of nucleotide synthesis, cofactor synthesis, amino acid synthesis, acetamide metabolism, proline metabolism, sulfate metabolism, and nitrate metabolism.

11. The method of claim 9, wherein the selective marker is a gene selected from the group consisting of *argB* (ornithine carbamoyltransferase), *amdS* (acetamidase), *bar* (phosphinothricin acetyltransferase), *hemA* (5-aminolevulinate synthase), *hemB* (porphobilinogen synthase), *hygB* (hygromycin phosphotransferase), *niaD* (nitrate reductase), *prn* (proline permease), *pyrG* (orotidine-5'-phosphate decarboxylase), *pyroA*, *riboB*, *sC* (sulfate adenyltransferase), and *trpC* (anthranilate synthase).

12. The method of any of claims 1 to 11, wherein the replication initiating sequence is a nucleic acid sequence selected from the group consisting of:

(a) a replication initiating sequence having at least 50% identity with the nucleic acid sequence of SEQ ID NO:1 or SEQ ID NO:2 and is capable of initiating replication;

(b) a replication initiating sequence which hybridises under low stringency conditions with (i) the nucleic acid sequence of SEQ ID NO:1 or SEQ ID NO:2, or (ii) the respective complementary strands, wherein the low stringency conditions are defined by prehybridization and hybridization at 42°C in 5x SSPE, 0.3% SDS, 200 mg/ml

sheared and denatured salmon sperm DNA, and 25% formamide, and wash conditions are defined at 50°C for 30 minutes in 2x SSC, 0.2% SDS; and

(c) a subsequence of (a) or (b), wherein the subsequence has replication initiating activity.

13. The method of claim 12, wherein the nucleic acid sequence has at least 50% identity, more preferably about 60%, even more preferably about 70%, even more preferably about 80%, even more preferably about 90%, and most preferably about 97% identity with the nucleic acid sequence of SEQ ID NO:1 or SEQ ID NO:2.

14. The method of claim 12, wherein the replication initiating sequence is obtained from a filamentous fungal cell.

15. The method of claim 14, wherein the filamentous fungal cell is a strain of *Aspergillus*.

16. The method of claim 15, wherein the strain of *Aspergillus* is obtained from a strain of *A. nidulans*.

17. The method of any of claims 12 to 16, wherein the replication initiating sequence has the nucleic acid sequence set forth in SEQ ID NO:1 or SEQ ID NO:2, or is a respective functional subsequence thereof.

18. The method of claim 2, wherein the modification of the parent polynucleotide sequence is performed by mutagenesis, preferably random mutagenesis, by use of a physical or chemical mutagenizing agent, use of a doped oligonucleotide, DNA shuffling, or by subjecting the nucleic acid sequence to

PCR generated mutagenesis, or use of any combination thereof.

19. The method of claim 18, wherein the polynucleotide sequences of interest are obtained by in vivo recombination between two or more homologous nucleic acid sequences encoding a polypeptide or a regulatory sequence, or any combination of both, comprising:

(a) identifying at least one conserved region between the polynucleotide sequences of interest;

(b) generating fragments of each of the polynucleotide sequences of interest, wherein said fragments comprise the conserved region(s) of (a); and

(c) recombining the fragments of (b) by using the conserved region(s) as (a) homologous linking point(s).

20. The method according to any of claims 1-19, wherein the filamentous fungal cell transformed with the population of DNA vectors is a cell of a strain of *Acremonium*, *Aspergillus*, *Coprinus*, *Fusarium*, *Humicola*, *Mucor*, *Myceliopthora*, *Neurospora*, *Penicillium*, *Thielavia*, *Tolypocladium* or *Trichoderma*.

21. The method according to claim 20, wherein the cell is an *Aspergillus oryzae*, *Aspergillus niger*, *Aspergillus nidulans*, *Coprinus cinereus*, *Fusarium oxysporum*, or *Trichoderma reesei* cell.

22. A method of constructing and screening or selecting a library of polynucleotide sequences of interest in a filamentous fungal cell, wherein the method comprises:

(a) transforming a culture of bacterial or yeast cells with a population of the DNA vectors as described in any of claims 1-21, wherein the vector further comprises a nucleic acid sequence encoding a bacterial or yeast selective marker and a bacterial or yeast replication initiating sequence;

(b) cultivating the bacterial or yeast cells under selection pressure;

(c) isolating the DNA constructs from the transformants of (b);

(d) transforming filamentous fungal cells with the DNA constructs of (c);

(e) cultivating the filamentous fungal cells of (d);

(f) selecting or screening for one or more filamentous fungal transformants expressing a desired characteristic; and

(g) isolating the filamentous fungal transformant(s) of interest.

23. The method of claim 22, wherein the bacterial or yeast selective marker is selected from the group of genes which encode a product which provides for resistance to biocide or viral toxicity, resistance to heavy metal toxicity, or prototrophy to auxotrophs.

24. Use of a fungal replication initiating sequence in the construction of a library of polynucleotide sequences of interest.

25. The use according to claim 24, wherein the fungal replication initiating sequence is a nucleic acid sequence selected from the group consisting of:

(a) a replication initiating sequence having at least 50% identity with the nucleic acid sequence of SEQ ID NO:1 or SEQ ID NO:2 and is capable of initiating replication;

(b) a replication initiating sequence which hybridises under low stringency conditions with (i) the nucleic acid sequence of SEQ ID NO:1 or SEQ ID NO:2, or (ii) the respective complementary strands, wherein the low stringency conditions are defined by prehybridization and hybridization at 42°C in 5x SSPE, 0.3% SDS, 200 mg/ml sheared and denatured salmon sperm DNA, and 25% formamide, and wash conditions are defined at 50°C for 30 minutes in 2x SSC, 0.2% SDS; and

(c) a subsequence of (a) or (b), wherein the subsequence has replication initiating activity.

26. The use according to claim 25, wherein the replication initiating sequence is obtained from a filamentous fungal cell, in particular from a strain of *Aspergillus*, such as *A. nidulans*.

27. The use according to claim 25 or 26, wherein the replication initiating sequence has the nucleic acid sequence set forth in SEQ ID NO:1 or SEQ ID NO:2, or is a respective functional subsequence thereof.

28. A library of polynucleotide sequences of interest which library comprises filamentous fungal cells tranformed with a population of DNA vectors, wherein each vector comprises:

(i) a gene encoding a fungal selection marker and a fungal replication initiating sequence wherein the marker and

the replication initiating sequence do not vary within the population; and

(ii) a polynucleotide sequence of interest wherein the population of DNA vectors contains more than one variant of the polynucleotide sequence.

29. The library according to claim 28, wherein the vector further comprises a nucleic acid sequence encoding a bacterial or yeast selective marker and a bacterial or yeast replication initiating sequence.

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